Ferns: Potential In-situ Bioassay Systems for Aquatic-borne Mutagens

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It is now apparent that cancer constitutes one of the leading health problems in the United States. A major investment of the energies and resources of the scientific community is being made to understand something of the causes of cancer as well as its cures. Parallel, and in many cases included, with these studies are investigations into the mechanisms of mutation of the genetic material (DNA), which, given the heritable nature of the cancerous state in a given cell line, may shed light on the mechanism of cancer induction and growth (Freese, 1971).

Equally apparent in recent years is the increasing introduction of man-made chemicals into our environment, either by their disposal in industrial wastes or via their use in pesticides, plastics, food additives, drugs, etc. Environmental groups raise a cry of warning against these pollutants, while biochemists show that an increasing number of the chemicals now present in our soil, air, water, food, and manufactured products are chemical carcinogens and mutagens. The debate has thus begun as to the benefits and risks of using such chemicals. But in order for the crucial debate to be meaningful, much precise information must be collected. Various methods are needed to assay the impact of technology on biology, both in the laboratory and in the field, using rapid microbial systems, critical mammalian systems, and on-site monitoring of native plant and animal populations.

Plant geneticists and cytogeneticists can contribute their study of mutations to the problems of cancer and environmental carcinogenesis. The value to cancer research and cancer prevention of studying mutations and mutagens has been demonstrated in recent years by the significant correlation between the cancer-inducing properties of various chemicals and their capacity to induce mutations in DNA in a wide variety of organisms (Miller & Miller, 1971; Ames et al., 1973, 1975). To quote from the Millers' survey: "In summary, it appears that many, perhaps all, chemical carcinogens are potential mutagens. Similarly, many, but possibly not all, mutagens are potential carcinogens." Test systems used to establish these correlations have utilized organisms ranging from viruses, bacteria, fungi, angiosperms, and fruit flies to mammals; even extracts from mammalian tissue are tested in the more practical and efficient bacterial systems. A wide survey of carcinogens, many of them known human carcinogens, in one such bacterial system found that 90% (156/174) of carcinogens tested are also mutagenic (McCann, et al., 1975).

The presence and activity of these and other suspect chemicals in our environment must be detected and analyzed. Such screening and subsequent elimination from the environment may provide the major means of cancer prevention. We are allowing ourselves to be exposed increasingly to chemical carcinogens and muta-

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gens that are being more strongly implicated as factors initiating most human cancer (Cairns, 1975; Epstein, 1974; Higginson, 1969). Cairns (1975) said that "a substantial proportion of cancer deaths could be prevented by controlling appropriate constituents of the environment." The problem then, to a great extent, becomes one of characterizing mutagens/carcinogens and detecting their presence and activity in the environment. The screening of entire ecosystems for the presence of mutagens may become a tractable problem if the appropriate bioassay systems are available. One method of approaching this problem is to measure mutation rates in some component of the flora of a given ecosystem. For example, the detection of increased mutation rates in certain species of an aquatic ecosystem which is characterized by the presence of certain kinds of industrial pollution certainly would warrant further laboratory testing of the pollutants for mutagenic activity. The value of in-situ plant bioassay systems for mutagens in these situations is that such bioassays serve as continuous and cumulative monitors, and so long periods of time are screened. Samples of water or undiluted effluent will vary in their mutagenicity since given mutagens resulting from industrial pollution vary as industrial production and methods change during the course of time. In contrast, the mutations of a submersed or semi-submersed plant species in a riparian ecosystem should reflect the cumulative genetic damage that has occurred during a long period of time. This period of time may be a single growing season or many years, depending upon the plant. In some bioassays it may be possible even to date when mutation rates changed; this refinement requires a detailed knowledge of the organography and ontogeny of the plants. This paper discusses a fern bioassay system with which a riparian ecosystem was screened for mutagenic activity and an attempt made at dating the mutational events.

For the past seven years, the senior author has been developing experimental techniques for the detection of genetic variability in fern populations (Klekowski, 1970, 1973). Recently these techniques have been applied to fern populations growing in environments that are polluted heavily with industrial wastes (Klekowski, 1975, 1976; Klekowski & Berger, 1976). These techniques, based on the Royal Fern, *Osmunda regalis* var. *spectabilis* (Willd.) Gray, involve the detection of both gene and chromosome mutations and whether such mutations have been induced post-zygotically in the parental sporophyte. The Royal Fern is admirably suitable for storing environmentally induced mutations. It has rhizome apices and leaf primordia based upon single apical cells that divide and give rise to all subsequent cells of a given organ. The induction of a mutation in these apical cells results in the mutation being passed to all subsequent cells of the organ. Therefore, as long as the mutations are not dominant cell lethals, they would be expected to accumulate in the genotypes of those organs as the organism grows and as mutations occur. Dominant deleterious mutations, involving those loci which control meiosis and aspects of early embryogeny, as well as recessive and dominant mutations at those loci involved in spore development, gametophyte maturation, gametangia development, zygotic development, and early embryogeny may be expected to accumulate in the apical cells of these organisms. Techniques are available to screen the genotype of a sporophyte for just such

mutations (Klekowski, 1971). Spore samples obtained from single sporophylls of a given sporophyte can be used to generate cultures of gametophytes in the laboratory. The frequency of viable and inviable spores can be scored readily. The gametophytes can be scored for normal and abnormal morphology, and the former used in genetic experiments to determine whether their genotypes contain recessive or dominant zygotic or sporophytic lethals. Techniques to do this involve the establishment of cultures which have one gametophyte each. Such gametophytes become hermaphroditic, forming both antheridia and archegonia simultaneously, and self-fertilization results in formation of homozygous zygotes from which homozygous sporophytes develop. Such sporophytes can be scored for normal or abnormal development.

Meiotic samples taken from the same sporophytes can be screened for chromosome mutations. It is fortunate that in *O. regalis* var. *spectabilis* very large chromosomes are present at meiosis in spite of the fact that $2n=44$. Two types of chromosome aberrations can be detected readily, both of which require two chromosome breaks for their origin. Reciprocal translocations can be detected at meiosis by the presence of multivalents (associations of four chromosomes at metaphase I or trivalents and univalents). It is the latter configuration which is detected most easily as the univalents very often fail to align in the metaphase I plate and with subsequent divisions of meiosis are left in the cytoplasm as micronuclei. Thus a given meiotic sample can be screened readily for the presence or absence of these micronuclei. Where micronuclei occur, further investigation of metaphase I and the pre-metaphase I stages of meiosis reveals the presence of multivalents. Another chromosome mutation that can be screened for in the meiocytes of this fern is the presence of paracentric inversions. Such chromosome mutations result in the formation of bridges and acentric fragments at anaphase I or anaphase II of meiosis. The occurrence of these configurations is based upon the position of cross-overs in the bivalent containing the inverted segment. The presence of bridges and acentric fragments in a sample of meiocytes can be taken as evidence of inversion heterozygosity (see Klekowski and Berger, 1976, for further discussion on the detection of chromosome aberrations in ferns).

In New England the Royal Fern is found commonly in most moist habitats, swamps, and bogs, and very often occurs as a component of the riparian flora. In the latter cases, the fern grows at the edges of watercourses, and very often its rhizomes are submersed periodically. The fern occurs only in rivers where silting is not a normal situation. The Royal Fern population which we investigated grows along the Millers River below Erving, Massachusetts.

The Millers River, a tributary of the Connecticut River, is approximately 50 miles long and drains 300 square miles of north central Massachusetts and 70 square miles of southern New Hampshire. Along its length the quality of the mainstem and its tributary the Otter River varies and is classified from B (good) to D (poor) based on a scale of the Massachusetts Division of Water Pollution Control. The 1.5 mile portion in which the *Osmunda* population grows is classified currently as D. The population consists of approximately 100 plants along the south bank of the river one mile below the outfall of the Erving Paper Com-

pany, Inc. Many of the plants have their rhizomes and shoot apices submersed most of the year. Fronds are initiated below the surface and emerge from the water as the fiddleheads uncoil. Only submersed plants were studied genetically.

Meiotic samples collected in the spring of 1973 from the Millers River population revealed that approximately 43% were heterozygous for chromosome mutations such as paracentric inversions and reciprocal translocations, whereas less than 1% of meiotic samples collected from nearby, non-polluted control populations gave evidence of such mutational heterozygosity. These control populations were taken from areas within the Millers River watershed.

Further analysis of the chromosome mutations present in the Millers River population was undertaken in the spring of 1974. Meiotic collections were made to determine the nature of the cytogenetic chimeras present within the sporophytes. The patterns of chromosome mutations were analyzed in an effort to date the time of induction. After extensive analysis, it was found that practically all the chromosome mutations detected in 1973 and 1974 represented mutations that had occurred since the sporophytes were growing in the Millers River, i.e. were post-zygotic mutations. It was found also that 64% of these chromosome mutations were induced since 1969. Thus, it was concluded that the waters of the Millers River were active mutagenically.

The frequency of Royal Fern sporophytes which are chimeric for gametophytic and sporophytic lethals also was studied in both the Millers River and control populations. A hybridization program was designed to determine whether the several interconnected rhizome apices resulting from the continued growth of a single sporophyte were heterozygous for allelic lethals (for details see Klekowski, 1976). Where the genotypes of these apices differed with reference to these lethals, post-zygotic mutations have occurred. Approximately 40% of the sporophytes studied from the Millers River population were chimeric for such deleterious mutants, whereas chimeras were absent from the control population in the non-polluted environment. This value (40%) is remarkably similar to the frequency of sporophytes exhibiting chromosome mutations (43%) based upon the cytological investigation previously discussed. The similarity of the genetic and cytological studies suggests that both methodologies give useful estimates of the amount of post-zygotic mutational damage present in the population. Because of the laborious and expensive nature of the genetic investigations (due to the prolonged culture and maintenance of thousands of gametophyte cultures), it appears that cytological methods offer an easier method of detecting post-zygotic mutational damage in Royal Fern populations.

These studies of the Royal Fern suggest that other ferns growing in riparian situations may be useful for in-situ bioassay systems of water-borne mutagens resulting from industrial pollution. Species such as *Lorinseria areolata* (L.) Presl, *Matteuccia struthiopteris* (L.) Tod., *Onoclea sensibilis* L., and members of the genus *Acrostichum* have the appropriate ecologies. But whether these species have the appropriate sensitivity, as well as suitable cytological and cultural characteristics, must be investigated before their usefulness for mutagen bioassays can be determined.

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